REJECTIONS UNDER 35 USC §112

Rejections of claims 37, 50, 63 and 76: Claims 37, 50, 63 and 76 have been amended to indicate that the measuring device is for determining the dilution of a sample discharged from the column (see page 7, line 16-21 of the specification).

Rejections of claims 40, 54, 67 and 80: Claims 40, 54, 67 and 80 have been amended to indicate that an electronic control means controls elution of sample out of the column (see page 11, lines 10-11 of the specification).

Rejection of claims 43 and 69: Claims 42-54 and 69-80 a device and method for measuring the concentration of an enzyme inhibitor in liquids. In accordance with this aspect of the invention, enzyme in the sample is bound on the column and inhibitor is allowed to pass through the column. In order to be certain that all of the enzyme in a given sample is retained on the column, the chromatographic carrier must include an excess amount of substance capable of binding an enzyme relative to the amount of enzyme in the sample. Hence, claims 43 and 69 as pending are correct.

Rejection of claim 56: Claims 55-57 are directed to a method for measuring the activity of an enzyme in liquid. In accordance with this aspect of the invention, inhibitor in the sample is bound on the column and enzyme is allowed to pass through the column. In order to be certain that all of the inhibitor in a given sample is retained on the column, the chromatographic carrier must include an excess amount of substance capable of binding an enzyme inhibitor relative to the amount of inhibitor in the sample. Hence, claim 56 as pending is correct.

THE INVENTION

The present invention is directed to a device (claims 29-41) and method (claims 55-67) for continuously, automatically, and reproducibly determining enzyme activity in a sample. The claimed device includes a flow through column and a detector. The flow through column includes a chromatographic carrier having a substance capable of binding an enzyme inhibitor corresponding to the enzyme in the sample. Hence, the claimed method and device are effective for continuously, automatically, and reproducibly removing an enzyme inhibitor from a sample so that enzyme activity can be accurately measured.

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In another aspect, the invention is directed to a device (claims 42-54) and method (claims 68-80) for continuously, automatically, and reproducibly determining inhibitor concentration in a sample. The claimed device includes a flow through column and a detector. The flow through column includes a chromatographic carrier having a substance capable of binding an enzyme corresponding to the enzyme inhibitor in the sample. Hence, the claimed method and device are effective for continuously, automatically and reproducibly removing an enzyme from a sample so that enzyme inhibitor concentration can be accurately measured.

Rejections of claims 29-34, 38, 55-60 and 64 under 35 USC 103(a) over EP 329,190 in view of Koohmaraie et al.

EP 329190: EP '190 does not describe a flow through column where the column includes a chromatographic carrier having a substance capable of binding an enzyme inhibitor as claimed. The purpose of the EP '190 device is not to bind enzyme inhibitors. EP '190 describes a device that includes a column containing a substrate immobilized on a chromatographic support (column 2, lines 33–54). Enzyme that is introduced into the column acts on the immobilized substrate to form decomposition products. These decomposition products are eluted from the column and detected (column 7, lines 9-24). The column of the EP '190 device would have to be changed with every pass since the amount of enzyme in a sample would not be known, and an excess amount of substrate would be needed in order to accurately determine enzyme activity.

The device described in EP '190 can not accurately measure enzyme activity in a sample that includes an inhibitor. If inhibitor is present in a sample being analyzed by the device and method described in EP '190, inhibitor is never removed from the sample and enzyme activity will be decreased and not accurately measured. Hence, if an inhibitor is present in the sample, the device and method described in EP '190 can only determine enzyme activity if the total amount of enzyme in the sample is already know or a known amount of inhibitor is added (column 11, lines 3-14).

Koohmaraie et al.: The Koohmaraie reference does not describe a flow through column where the column includes a chromatographic carrier having a substance capable of binding an enzyme inhibitor as claimed. The Koohmaraie references describes a batch process

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where a sample is first allowed to react with S-carboxymethylated-papain-Sepharose for 2 hours at 2 to 4°C with constant mixing (column 1 of page 2364). After this two hour incubation, the resin is allowed to settle, supernate is eluted, and the resin is washed twice. The resulting supernate must then be manually further processed to determine enzyme activity.

One of ordinary skill in the art would have no motivation to combine EP '190 with Koohmaraie et al. One of ordinary skill in the art facing the problem of measuring enzyme activity in a sample that may contain an inhibitor would not modify the column described in EP '190 to include the batch resin system described in Koohmaraie et al.

i) Koohmaraie is a slow batch process. The batch process of Koohmaraie et al. requires 2 hours incubation of sample with S-carboxymethylated-papain-Sepharose. One of ordinary skill in the art would not expect that a batch process that requires a 2 hour incubation with constant mixing would work as flow through column. The residence time on a flow through column would be far shorter than 2 hours and the mixing would not be as extensive. Hence, given the Koohmarie reference, one of ordinary skill would be more likely to conclude that a batch process is necessary and that a flow through system would not work to remove enzyme inhibitor.

ii) Koohmaraie requires refrigeration. The batch process described in Koohmaraie et al. is conducted at low temperature, 2 to 4°C. In order to measure enzyme activity, the temperature would have to be raised. Hence, one of ordinary skill would not expect a refrigerated batch process to work in a higher temperature flow through column.

iii) The combination of references does not provide a continuous and automated device or method. Neither reference alone or in combination provides a device or method that would allow for a continuous and completely automated measurement of enzyme activity. As described above, EP '190 requires additional steps to accurately measure enzyme activity if inhibitor is present in the sample. Further, Koohmaraie requires additional sample manipulation and transfer after removal of inhibitor in order to determine enzyme activity. The additional steps described in both references decrease the accuracy of the analysis and require additional technician time to complete the same analysis that is provided by the claimed device and method.

Rejection of claims 42-47, 51, 68-73 and 78 under 35 USC 103(a) over EP 329,190 in view of Koohmaraie et al. and U.S. Patent No. 4,030,977 to Fujii et al .

The combination of EP '190, Koohmaraie and the '977 patent does not describe or suggest a device or method for measuring enzyme inhibitor concentration in a sample. None of the references either alone or in combination describe a flow through column having a chromatographic carrier capable of binding enzyme in a sample such that enzyme inhibitor passes through the column and is separated from the enzyme. The separation provided by the present invention results in a relatively clean sample containing inhibitor which allows for automated and convenient subsequent determination of inhibitor concentration.

EP '190: As discussed above, EP '190 describes a column that includes a substrate upon which the enzyme acts to release decomposition products. Hence, enzyme is not bound or retained on the EP '190 column but merely acts to degrade its substrate that is bound on the column.

Koohmaraie: As discussed above, Koohmaraie et al. describes a batch process that utilizes a resin complex that binds inhibitors, not enzymes.

U.S. '977: The '977 patent does not describe a device and method where enzymes remain bound on a column and inhibitors pass through the column for subsequent measurement by a detector. The '977 patent describes a column that includes an enzyme or enzyme inhibitor absorbed to a column (see column 5, lines 46-49). As described in Example 3 at column 8 and in column 6, lines 21-35, enzyme and inhibitor are bound on the column and then eluted separately. The '977 patent is very specific in its purpose as it is directed to isolating and purifying enzymes that are involved in the production and consumption of kinin (column 1, lines 25-37).

Hence, the combination of references would not teach one of ordinary skill trying to analyze enzyme inhibitors to provide a column that binds enzyme such that the inhibitor corresponding to the enzyme would pass through the column and provide a relatively pure sample for subsequent analysis.

Rejection of claims 39, 40, 53, 54, 66, 67, 79 and 80 under 35 USC 103(a) over EP 329,190 in view of Koohmaraie et al. and U.S. Patent No. 4,030,977 to Fujii et al and U.S. Patent No. 4,762,617 to Stevens et al .

The combination of the cited references does not provide a device and/or method for measuring enzyme activity or the concentration of enzyme inhibitor where the device includes a flow through column with the chromatographic carrier as claimed.

EP '190: As discussed above, EP '190 describes a column that includes a substrate upon which the enzyme acts to release decomposition products. Hence, enzyme is not bound or retained on the EP '190 column but merely acts to degrade its substrate that is bound on the column.

Koohmaraie: As discussed above, Koohmaraie et al. describes a batch process that utilizes a resin complex that binds inhibitors, not enzymes.

U.S. '977: The '977 is directed to purifying protein, not a device and/or method for measuring enzyme activity as claimed. As indicated at column 6, lines 3-6, "Generally, column chromatography is preferred when it is desired to obtain an eluate containing the desired product in a high concentration . . . ". Hence, the device and method described in the '977 patent would not be useful for reproducibly measuring enzyme activity in large numbers of patient fluids.

U.S. '617: The '617 patent does not describe a flow through column that includes a chromatographic carrier with a substance capable of binding an enzyme inhibitor corresponding to the enzyme being analyzed. The '617 patent is directed to a chromatography system employing size exclusion and gel filtration for analysis of macromolecules. Hence, the column described in the '617 patent does not bind anything but merely retains molecules due to physical size constraints.

Double Patenting

Applicants will provide a terminal disclaimer upon receipt of notice of allowable claims.

Conclusion

In view of the above-indicated amendments and remarks, Applicant respectfully requests that the pending claims be passed to issue.

Respectfully submitted,

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